Remarks

With entry of the amendment, claims 44-74 are pending and claims 1-43 are canceled. The Office Action of February 9, 2006 was based on then pending claims 1, 3-5, 21, 27, 28, 32-37, and 40. Claims 3-5, 21, 27, and 28 were objected to as depending from a canceled base claim and were withdrawn from consideration. Claim 40 was rejected under 35 U.S.C. 102(b); claims 1 and 32-37 were rejected under 35 U.S.C. 103(a); and claims 1, 16, and 27 were rejected under the judicially created doctrine of obviousness-type double patenting.

Telephonic Examiner Interview

In a May 23, 2006 telephone interview, Promega Patent Counsel Laura Bozek, Dr. Rex Bitner, and the undersigned discussed the invention with Examiners Christopher Gross and Mark Shibuya. Applicants wish to thank the Examiners for the courtesy of their time and attention to this application.

Applicants were invited to submit literature that compares prior extraction methods to methods of the instant invention. Kits according to the present invention used in methods of the invention are sold by Promega Corporation as DNA IQ™, which is described in the attached publication entitled "Application of the BioMek® 2000 Laboratory Automation Workstation and the DNA IQ™ System to the Extraction of Forensic Casework Samples", co-authored by Susan Greenspoon, Forensic Molecular Biologist with the Virginia Department of Forensic Science.

Claim amendments

The amendment introduces no new matter and is fully supported by the specification as indicated below.

Support for new claim 44 can be found at least in original claims 1 and 3, at page 6, lines 24-26, and at page 9, lines17-25 of the specification.

Support for new claim 45 can be found at least in original claims 1 and 3.

Support for new claim 46 can be found at least in original claims 1-3.

Support for new claims 47-54 can be found at least in original claims 1-12.

Support for new claim 55 can be found at least in original claim 13.

Support for new claim 56 can be found at least in original claim 14.

Support for new claim 57 can be found at least at page 7, line 11 of the specification.

Support for new claim 58 can be found at least at page 9, line 14 of the specification.

Support for new claim 59 can be found at least in original claim 15.

Support for new claims 60-64 can be found at least in original claims 16-19.

Support for new claim 65 can be found at least in original claim 26.

Support for new claim 66 can be found at least at page 9, lines 10-20 of the specification.

Support for new claim 67 and 68 can be found at least at page 9, lines 25-30 of the specification.

Support for new claim 69-74 can be found at least in original claims 32-37.

In view of the amendments above and arguments below, Applicants respectfully request allowance of claims 44-74.

Rejections under 35 USC 102(b)

Claim 40 is rejected under 35 USC 102(b) as being anticipated by Makowski *et al.* (1997 J. Clinical Laboratory Analysis 11:87-93), which the Office Action characterized as teaching isolating DNA from blood samples on solid supports impregnated with a chaotropic salt by incubating with 95°C water. Claim 40 has been cancelled, thus rendering moot the rejection.

Rejections under 35 USC 103(a)

Rejection of claims 1, 32, and 35-37 over Hornes *et al.* in view of Boom *et al.*

Claims 1, 32, and 35-37 are rejected under 35 USC 103(a) as being unpatentable over Hornes *et al.* (US Patent 5,512,439) in view of Boom *et al.* (J. Clinical Microbiology 28:495-503).

The Office Action characterized Hornes *et al.* as teaching a discrete quantity of a solid support capable of reversibly binding a defined quantity of DNA, while acknowledging that Hornes *et al.* fails to teach silica in the presence of a chaotrope. Boom *et al.* is cited as teaching silica beads for binding nucleic acids in the presence of a chaotrope. The Examiner concluded that one of ordinary skill in the art would have been motivated to combine the references to make the claimed invention because Boom *et al.* ostensibly permits isolation of DNA from clinical samples.

A *prima facie* case of obviousness requires: (1) some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) a reasonable expectation of success; and (3) the art reference or combination of references must teach all

of the claim limitations (MPEP 2142). The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) (MPEP 2143).

Applicants respectfully submit that none of claims 44-74 is *prima facie* obvious over Hornes *et al.* and Boom *et al.* because the references do not combine to teach all of the claim limitations. The claims require isolating a defined and consistent amount of DNA from multiple samples. Each sample, which comprises DNA in excess of the binding capacity of the discrete amount of silica-containing solid support, is contacted with a discrete amount of a silica-containing solid support such that a defined amount of DNA is reversibly bound to the solid support. Each sample is then separated from the support to isolate a defined and consistent amount of DNA from each sample.

One of skill in the art would understand from the specification that the claimed invention permits isolation of a uniform amount of DNA from multiple samples. In other words, when using a particular discrete amount of DNA to isolate DNA from each of multiple samples having DNA in excess of the binding capacity of the silica-containing solid support, one would obtain a consistent amount of DNA from each sample.

The importance of obtaining consistent amounts of DNA is discussed throughout the specification in the context of performing downstream applications. For example, at page 3, lines 1-21 of the specification, the problems of having excess DNA template in amplification reactions are discussed. Traditionally, these problems have been addressed by measuring DNA concentrations prior to use in downstream applications. However, measuring DNA concentrations consumes the DNA sample and is inaccurate for samples having low concentrations of DNA (page 4, lines 16-18).

As discussed throughout the specification, the ability to isolate a consistent amount of DNA from a sample permits the DNA thus isolated to be used directly in subsequent applications requiring DNA within any given range without first having to measure the concentration of DNA in order to determine the volume of purified DNA necessary to give an amount of DNA within a suitable range (page 5, lines 20-33; page 9, lines 10-33). This is particularly important when isolating DNA from relatively scarce sources, such as trace evidence.

Hornes *et al.* teaches superparamagnetic particles that carry a plurality of molecules of an oligonucleotide probe that specifically bind select single stranded DNA or RNA molecules by hybridizing to particular sequences. For example, particles derivatized with

polydeoxythymidine (oligo dT) can hybridize to the poly A tract of mRNA molecules (column 5, lines 42-46). Single stranded DNA can be isolated using particles derivatized to include a probe having a DNA sequence complementary to a known target sequence (column 7, lines 30-32). Applicants acknowledge that Hornes *et al.* discloses determining the capacity of beads derivatized with $(dT)_{25}$ to bind to oligo $(dA)_{25}$. However, Hornes *et al.* does not teach or suggest isolating a defined amount of DNA by contacting a silica containing solid support with a sample having DNA in excess of the binding capacity of the beads. Typically, hybridizations were conducted using approximately a two-fold excess of T-bead hybridization capacity to the complementary oligo (dA)25 target nucleotide (see Hornes et al., column 16, lines 58-61). The focus of Hornes *et al.* is on maximizing efficiency of recovery of specific target nucleic acids (col. 17, lines 1-20) rather than isolating a defined amount of nucleic acid. For example, Hornes *et al.* emphasizes that the T-beads bind at least 99% of the target oligonucleotides even when the molar ratio of target to bead capacity approaches 1:1 (col. 17, lines 1-7) or when the molar ratio of the target to bead capacity is nearly 1:1 (column 12, lines 37-40).

Furthermore, there is no suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine Hornes *et al.*, which teaches isolating particular target nucleic acids through specific binding (i.e., hybridization of two complementary nucleic acid sequences), and Boom et al, which teaches non-specific binding to silica of nucleic acids added to a substantially sterile biological sample (i.e., serum or urine). In fact, Hornes *et al.* teaches away from combining the references in that Hornes *et al.* expressly teaches and claims using particles having a coating that reduces non-specific binding (please see column 4, lines 29-31; claim 1). Clearly, one of skill in the art would appreciate that silica, which binds nucleic acids non-specifically, would not be an appropriate particle coating for use in isolating specific sequences according to the methods of Hornes *et al.* Thus, not only is there no motivation to combine the references, there is no reasonable expectation of success. A *prima facie* case of obviousness requires that the teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure.

Rejection of claims 1, 32, 33 and 35-37 over Hornes *et al.* in view of Gocke *et al.*

Claims 1, 32, 33, and 35-37 are rejected under 35 USC 103(a) as being unpatentable over Hornes *et al.* in view of Gocke *et al.* (US Patent No. 6,156,504). The Examiner acknowledged that Hornes *et al.* does not teach silica particles or liquid blood. Gocke *et al.* is

cited as teaching extraction of extracellular nucleic acid from blood plasma onto silica (col. 6, lines 20-27). The Examiner asserts that Gocke provides one of ordinary skill in the art with motivation to modify Hornes *et al.* because Gocke teaches that extracellular DNA contains cancer markers and, the Examiner reasoned, "one skilled in the art would have measured the oligonucleotide loading of the derivatized particles of Gocke *et al.* using the exacting radiometric precision demonstrated by Hornes *et al.* with a reasonable expectation of success since radiometric counting is well known in the art to be very precise."

As explained above, Hornes *et al.* does not teach or suggest isolating a defined and consistent amount of DNA from multiple samples having DNA in excess of the binding capacity of the silica-containing solid support by contacting each sample with a discrete amount of a silica-containing solid support and separating each sample from the support to isolate the defined amount of DNA, as required by claims 44-74. Applicants respectfully submit that, even though one of skill in the art may be motivated to isolate DNA from blood, Gocke *et al.* fails to cure the deficiency of the primary reference in that Gocke *et al.* does not teach isolating consistent amounts of DNA from multiple samples by contacting the samples with a discrete amount of silica-containing solid support, the samples having DNA in excess of the binding capacity of the silica-containing solid support.

Furthermore, there is no suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine Hornes *et al.*, which teaches isolating particular target nucleic acids through specific binding (i.e., hybridization of two complementary nucleic acid sequences), and Gocke *et al.* In fact, Hornes *et al.* teaches away from combining the references in that Hornes *et al.* expressly teaches and claims using particles having a coating that reduces non-specific binding (please see column 4, lines 29-31; claim 1). Clearly, one of skill in the art would appreciate that silica, which binds nucleic acids non-specifically, would not be an appropriate particle coating for use in isolating specific sequences according to the methods of Hornes *et al.* Thus, not only is there no motivation to combine the references, there is no reasonable expectation of success.

Rejection of claims 1 and 32-37 over Hornes *et al.* in view of Bienhaus

Claims 1 and 32-37 were rejected under 35 USC 103(a) as being unpatentable over Hornes *et al.* in view of Bienhaus (US Patent No. 5,746,978). The Examiner, citing Bienhaus at column 2, lines 6 and 45 and column 3, line 1 and claim 34, asserted that Bienhaus teaches

a closed device for extracting nucleic acids from blood and other materials, including solids via silica beads.

As explained above, Hornes *et al.* does not teach or suggest isolating a defined and consistent amount of DNA from multiple samples having DNA in excess of the binding capacity of the silica-containing solid support by contacting each sample with a discrete amount of a silica-containing solid support and separating the samples from the support to isolate the defined amount of DNA, as required by claims 44-74. Applicants respectfully submit that, even though one of skill in the art may be motivated to isolate DNA from blood, Bienhaus fails to cure the deficiency of the primary reference in that Bienhaus does not teach isolating DNA by contacting a silica-containing solid support with a sample having DNA in excess of the binding capacity of the silica-containing solid support.

Furthermore, there is no suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine Hornes *et al.*, which teaches isolating particular target nucleic acids through specific binding (i.e., hybridization of two complementary nucleic acid sequences), and Bienhaus *et al.*, which teaches non-specific binding to silica of nucleic acids added to a substantially sterile biological sample (i.e., serum or urine). In fact, Hornes *et al.* teaches away from combining the references in that Hornes *et al.* expressly teaches and claims using particles having a coating that reduces non-specific binding (please see column 4, lines 29-31; claim 1). Clearly, one of skill in the art would appreciate that silica, which binds nucleic acids non-specifically, would not be an appropriate particle coating for use in isolating specific sequences according to the methods of Hornes *et al.* Thus, not only is there no motivation to combine the references, there is no reasonable expectation of success.

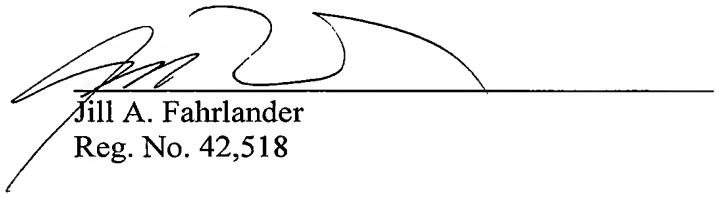
Rejection of claims under the judicially created doctrine of obviousness-type double patenting

Claims 1 and 40 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1, 16, and 27 of U.S. Patent No. 6,673,631. This is obviated by the enclosed terminal disclaimer, executed by the undersigned attorney of record.

In light of the foregoing, Applicants submit that the claims are in condition for allowance, and respectfully request notification to that effect. Should the Examiner feel that anything warrants further discussion, the Examiner is encouraged to contact the undersigned at the phone number below.

Please charge Deposit Account No. 50-0842 with any fees owed in connection with this submission.

Respectfully submitted,



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Application of the BioMek® 2000 Laboratory Automation Workstation and the DNA IQ™ System to the Extraction of Forensic Casework Samples*

ABSTRACT: Robotic systems are commonly utilized for the extraction of database samples. However, the application of robotic extraction to forensic casework samples is a more daunting task. Such a system must be versatile enough to accommodate a wide range of samples that may contain greatly varying amounts of DNA, but it must also pose no more risk of contamination than the manual DNA extraction methods. This study demonstrates that the BioMek® 2000 Laboratory Automation Workstation, used in combination with the DNA IQ™ System, is versatile enough to accommodate the wide range of samples typically encountered by a crime laboratory. The use of a silica coated paramagnetic resin, as with the DNA IQ™ System, facilitates the adaptation of an open well, hands off, robotic system to the extraction of casework samples since no filtration or centrifugation steps are needed. Moreover, the DNA remains tightly coupled to the silica coated paramagnetic resin for the entire process until the elution step. A short pre-extraction incubation step is necessary prior to loading samples onto the robot and it is at this step that most modifications are made to accommodate the different sample types and substrates commonly encountered with forensic evidentiary samples. Sexual assault (mixed stain) samples, cigarette butts, blood stains, buccal swabs, and various tissue samples were successfully extracted with the BioMek® 2000 Laboratory Automation Workstation and the DNA IQ™ System, with no evidence of contamination throughout the extensive validation studies reported here.

KEYWORDS: forensic science, BioMek® 2000, DNA IQ™, forensic casework, STR, DNA extraction

Any robotic system utilized to automate the extraction of evidentiary forensic casework samples must generate high quality nuclear DNA with yields comparable or better than those produced using manual extraction methods, particularly if limited amounts of DNA are present. One of the biggest challenges in adapting a robotic system for casework extraction is to provide a method flexible enough to handle the wide variety of samples which the forensic scientists routinely encounter. It is to be expected that such a system will be challenged with a wide range of samples that may contain greatly varying amounts of DNA. Moreover, the robotic system must not pose any greater risk of introducing contamination than manual DNA extraction methods. Robotic forensic sample DNA extraction has successfully been employed for database sample extraction (1), but has only recently been reported for the extraction of evidentiary type samples (2). The use of automated DNA extraction in clinical settings is more widely employed (3,4).

The DNA IQ™ System (Promega Corp., Madison, WI) employs a silica coated paramagnetic resin that binds DNA with high affinity in the presence of chaotropic agents such as guanidinium (5). Because no centrifugation or filtration steps are needed with this system, it readily lends itself to the application of "hands off" automated extraction. Cells are briefly lysed either in a Proteinase K containing buffer, then loaded into a 96 well plate on the robot or cells are lysed in the DNA IQ™ lysis buffer prior to loading onto the robot. The BioMek® 2000 Laboratory Automation Workstation (Beckman Coulter, Fullerton, CA) utilized in this study has been modified by Promega Corporation scientists for the application of the DNA IQ™ System (Fig. 1). A flow chart depicting the processing of the samples using the DNA IQ™ System is depicted in Fig. 2. The most notable features added by the Promega scientists are a magnetic plate on the deck (MagnaBot), a shaking platform attached as a right side module (DPC, Los Angeles, CA) and a thermal exchange unit which sits atop the shaking platform, attached to a water bath by tubing.

An exhaustive array of studies was performed during the optimization and evaluation of the BioMek® 2000 robot in conjunction with the DNA IQ™ System (BioMek® 2000/DNA IQ™ System). Sexual assault (mixed samples), cigarette butts, blood stains, buccal swabs, and various tissue samples were successfully extracted with the BioMek® 2000/DNA IQ™ System. Once extracted, the DNA yields were measured and the quality of the DNA assessed using either the PowerPlex® 1.1 (containing the CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, and D5S818 loci) or the PowerPlex® 16 BIO System (containing the FGA, TPOX, D8S1179, vWA, Amelogenin, Penta E, D18S51, D21S11, TH01, D3S1358, Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818 loci). The BioMek® 2000/DNA IQ™ System successfully extracted a large variety of samples with little evidence of contamination throughout the extensive validation studies reported here.

Materials and Methods

Software Methods

The software method used was the same whether or not the samples were sexual assault (mixed stain) samples that required

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* Portions of this work were presented at the 12th International Symposium on Human Identification.
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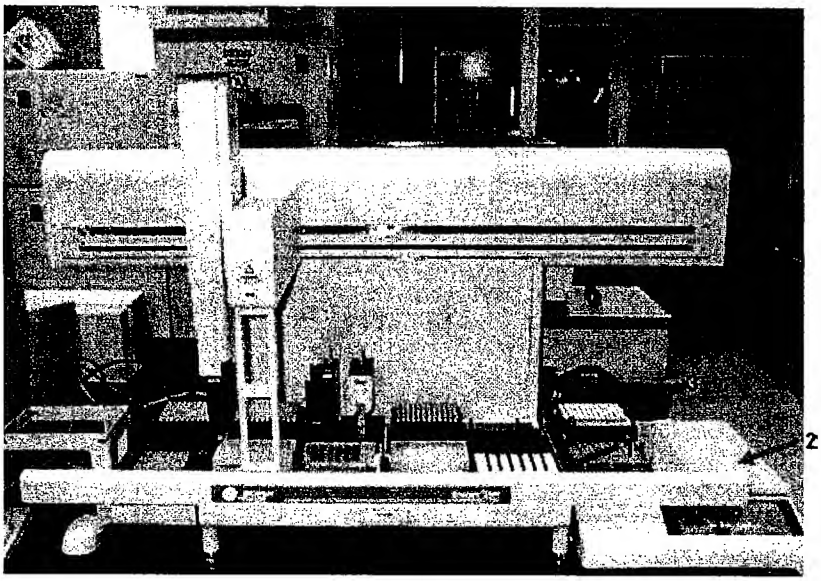

FIG. 1—*BioMek® 2000 robot with modifications for the application of the DNA IQ™ System for DNA extraction. Numbers and arrows refer to specific additions. 1 = magnet (Magnabot), 2 = shaking platform, 3 = thermal exchange unit.*

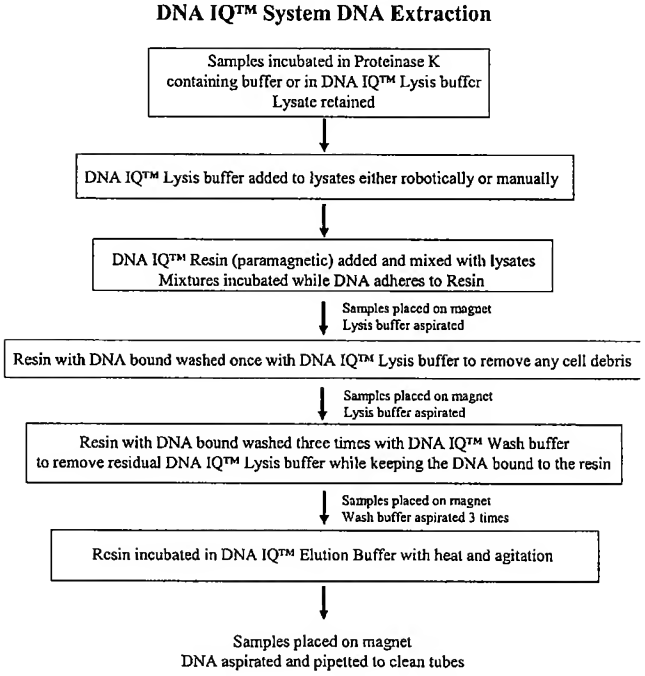

FIG. 2—*Flow chart depicting steps involved in the extraction of DNA using the DNA IQ™ System.*

a differential extraction, blood stains, cigarette butts, buccal swabs, or skin cells. The earliest version of the software method did not provide automated resin addition and contained an initial shaking step with samples loaded into a shallow Greiner plate. Initial contamination tests, reported in the Results Section, demonstrated a low level of contamination introduced during the robotic extraction process. Following these results, the software method was modified to include automated resin addition, as well as the use of a 96 deep well plate and the replacement of the initial shaking step with a pipetting step. This modified version of the software became the standard and was utilized for the bulk of the validation and optimization studies reported here. Versions of this software method were employed throughout this study which differed only in the numbers of samples (i.e., 24 versus 56 samples) the system processed, while keeping the DNA extraction process identical. All software methods were devised by Promega Corporation using Beckman Coulter software installed on the BioMek® 2000 robot.

Sample Preparation

Dried blood stain samples used for the contamination studies were created from liquid blood samples donated by Virginia Division of Forensic Science (VDFS) laboratory staff. Liquid blood samples were deposited on filter paper (Whatman, Clifton, NJ) or cotton swatches and stored at −20°C or at room temperature. The oldest samples used in this study were eight years old.

For the sensitivity study, freshly drawn liquid blood from three volunteers was deposited on blood stain cards (Whatman) in the following dilutions: neat, 1:10, 1:100, 1:1,000 and 1:10,000. Blood was diluted with sterile water prior to pipetting onto the blood stain cards. The cards were then air dried.

Tissue samples used for a contamination study and a tissue extraction study were previously obtained from the Office of the Chief Medical Examiner for the Commonwealth of Virginia and stored at −20°C.

Mock sexual assault samples were prepared from vaginal swabs and semen previously donated by VDFS employees. The semen was placed onto swabs undiluted and with dilutions at 1:2 and 1:4, in sterile water. A one-fourth (1/4) portion of each swab was removed for DNA extraction. A larger, comparative study was conducted in which the performance of the BioMek® 2000/DNA IQ™ System extraction of the non-sperm lysate and the sperm pellet was compared with manual organic extraction. For this study, five sets of mock sexual assault samples (referred to as "couples") were created. Eight vaginal swabs from the same donor were used for

each mock sexual assault set of samples and the semen was donated by one individual. Four dilutions were made with the semen, each dilution in a large enough volume (approximately 300 μL) to accommodate two vaginal swabs. Thus, each dilution was soaked into two vaginal swabs, thereby rendering two nearly identical sets of four samples from one "couple." Three of the five mock sexual assault "couples" had the following semen dilutions placed onto the vaginal swabs: 1:10, 1:100, 1:1,000 and 1:10,000. The fourth "couple" had the following semen dilutions: 1:10, 1:100, 1:200 and 1:400 placed onto the vaginal swabs. The last mock "couple" had the following semen dilutions: 1:100, 1:200, 1:400 and 1:800 placed onto the swabs. The mock sexual assault swabs were then air dried until use.

For the substrate and inhibitory substances study, blood was drawn from two volunteers and deposited on a variety of different surfaces. To test for inhibitory substances, the questioned substances were placed onto clean cotton prior to the deposition of blood. The different substrates were: canvas, blue jean, carpet, black underwear, hosiery, and a sanitary bag (with plastic liner). The possible inhibitory substances placed onto cotton were: motor oil, hand cream, hand soap, contraceptive foam, and dirt. Approximately 200 μL of blood was deposited onto each substrate and the samples allowed to dry before DNA extraction. Once the samples dried, a 5 mm^2 portion was removed for DNA extraction. This study also appears in another report (6).

For the environmental study, blood samples collected from three volunteers of the VDFS were applied to blood stain cards (Whatman) and subjected to the following environmental conditions for one day, one week, one month and three months: room temperature, moist at room temperature, 37°C, 56°C, 80°C, and sunlight at room temperature. This study also appears in another report (6).

Presumptive Test for Blood

A combined Phenolphthalein-Tetramethylbenzidine (PTMB) color test was utilized to assay for the presence of blood and was performed as described in the VDFS procedure manual which was adapted from several published studies (7–9). A positive result was indicated by a pink color (the Phenolphthalein) followed by an immediate blue-green color (the Tetramethylbenzidine). A negative result was indicated by a lack of pink color formed followed by no blue-green color. An inconclusive result was indicated by the development of color combinations other than those specified for a positive reaction, including one testing positive and the other testing negative.

DNA Quantitation, STR Typing and Analysis

Once extracted, all DNA samples were quantitated using the QuantiBlot™ Human DNA Quantitation kit (Applied Biosystems, Foster City, CA) using the chemiluminescent detection method (ECL reagent, Amersham, Piscataway, NJ), according to the manufacturer's recommendations.

Short tandem repeat (STR) amplifications were performed for most DNA samples using the PowerPlex® 1.1 System multiplex kit (Promega Corporation, Madison, WI). DNA samples for the substrate/inhibitory substances, the environmental study, and a portion of the sensitivity study were typed using the PowerPlex® 16 BIO System multiplex kit (Promega Corporation). DNA amplifications were performed using 1.0 ng of DNA in a volume of 10 μL. If less than 1.0 ng/10 μL of DNA was available for amplification, then the maximum volume (10 μL) in accordance with the Virginia Division of Forensic Science Procedures Manual was placed into the amplification reaction.

Prior to typing on an acrylamide gel, PCR products were initially visualized by electrophoresis in a 3% NuSieve® 3:1 (BioWhittaker Molecular Applications, Rockland, ME) agarose gel containing Ethidium Bromide to assess the quality and amount of amplified product produced. PowerPlex™ 1.1 PCR products were electrophoresed in a 6% Gel-Mix (Invitrogen, Carlsbad, CA) polyacrylamide gel for 2 h at 50 W. PowerPlex® 16 BIO PCR products were electrophoresed in a 6% Page Plus™ (Amresco, Solon, OH) polyacrylamide gel for approximately 2 h at 60 W.

After electrophoresis, both the PowerPlex® 1.1 and 16 BIO PCR products were visualized using the Hitachi FMBIO® II (MiraiBio, Alameda, CA). The scanning parameters used were those recommended in the PowerPlex® 1.1 and 16 BIO System Technical Manuals. Gel images were analyzed and allele sizing accomplished with the use of the FMBIO® Analysis and STaRCall™ software programs, respectively.

Contamination Studies

Sample cuttings, approximately 5 mm^2, were removed from each blood stain and incubated in 100–150 μL of the DNA IQ™ Lysis buffer for 30 min at 95°C. The first plate format utilized was dubbed the "Zebra Stripe" test. Samples containing a concentrated source of DNA (the blood stain lysates) were loaded into the wells of a single column in a plate, the wells of the adjacent column contained nothing but reagent blanks and the wells of the column adjacent to the reagent blank column were loaded with blood stain lysates. This alternating column pattern was repeated across the plate. The initial two contamination tests and the first differential extraction test utilized the original version of the software method for DNA extraction using the BioMek® 2000/DNA IQ™ System (described above), which required manual resin addition and volume reduction of the samples prior to transferring samples into a shallow Greiner plate which was then placed onto the robot deck for the robotic extraction. After the first two Zebra Stripe tests, the software method was modified as described (Software Method section) and samples were loaded into a 96 deep well plate for repeat testing of the Zebra Stripe test. All of the following tests were performed using the modified method.

The second plate format designed to readily detect contamination was referred to as the "Checkerboard" test. Samples containing a concentrated source of DNA, also in the form of blood stain lysates, were loaded into wells in an alternating fashion with reagent blanks. This pattern was continued across the plate so that the plate resembled a checkerboard. The well adjacent to a blood stain lysate sample, both horizontally and laterally, was always a reagent blank well.

Differential Extraction Studies

The preliminary test to determine if the BioMek® 2000/DNA IQ™ System could be incorporated into the differential extraction process utilized semen dilutions (described in Materials and Methods). Twenty-five microliters of semen were used for the undiluted control. The semen was placed onto previously collected vaginal swabs that had been stored at −20°C. The differential extraction procedure was performed according to the VDFS procedure that relies on previously published techniques (10), until the point at which a sperm pellet was generated and washed three times. This

initial test employed the original software method, described above, according to the manufacturer's directions. To the washed sperm pellet and epithelial cell (E-cell) lysate, DNA IQ™ Lysis buffer plus DNA IQ™ Resin were added, the samples then vortexed a few seconds and the tubes containing the suspensions transferred to a magnet stand. The liquid of all fractions was removed and discarded since the DNA was bound to the resin. Additional DNA IQ™ Lysis buffer was added, the samples vortexed, then transferred to a Greiner plate. Subsequently, the Greiner plate was placed onto the deck of the BioMek® 2000 robot and the extraction completed using the BioMek® 2000/DNA IQ™ System.

The newly modified version of the software (described in the Software Method section) replaced the original version of the software and a comparative study was performed to judge the performance of the BioMek® 2000/DNA IQ™ System in comparison with the manual organic extraction followed by ethanol precipitation. Five sets of mock sexual assault samples (each set referred to as a "couple") were created in duplicate as described above in the Sample Preparation section. Four semen dilutions were tested for each mock sexual assault "couple set" (e.g., 1:10, 1:100, 1:1,000, and 1:10,000). Thus, for each dilution, two nearly identical samples were placed into separate tubes for the differential extractions. The differential extraction procedure was performed manually as described above until the point at which separate epithelial (non-sperm) cell fractions were generated and the corresponding sperm pellets washed three times. The sample tubes were not designated as to whether the extraction would be completed robotically or manually until after the epithelial cell lysis in order to prevent any bias. Once the epithelial cell lysates had been transferred to another tube and the sperm pellets washed three times, the tubes were divided into two equivalent sets containing all the semen dilutions and the corresponding epithelial cell fractions. For each of the five "couple" sets of duplicate semen dilutions, half the set was extracted manually by a scientist in the laboratory using the mixed stain procedure for sperm cell lysis followed by the organic extraction and ethanol precipitation procedure as described in the VDFS procedure manual and the other half of the set was placed into a 96 deep well plate for BioMek® 2000/DNA IQ™ System extraction.

All sample sets that were completed using the BioMek® 2000/DNA IQ™ System had the entire sperm pellet and 1/5 the volume of the epithelial cell lysate (100 μL) loaded into the 96 deep well plate. The use of only 100 μL of the epithelial cell lysate was due to the volume limitations imposed by the software method employed by the BioMek® 2000/DNA IQ™ System: 100 μL for samples in a buffer other than the DNA IQ™ Lysis buffer and 150 μL for samples in the DNA IQ™ Lysis buffer. A small variation was made on the manual extraction of the samples after the first mock sexual assault "couple" extraction. Once the samples had been ethanol precipitated, they were re-suspended in 100 μL of TE^{-4} [TE^{-4}, 10 mM Tris buffer (pH 7.5), 0.1 mM EDTA] instead of 36 μL (as described in the VDFS procedure manual). This was designed to make it easier to compare DNA yields and performance since the BioMek® 2000/DNA IQ™ System at that time eluted the DNA from the resin into 100 μL of elution buffer.

Sensitivity Study

A blood stain square of approximately 5 mm^2 was cut in triplicate from each of the blood stain dilutions. One 5 mm^2 portion from each of the blood stain dilutions was extracted using the BioMek® 2000/DNA IQ™ System, one was extracted using the manual DNA IQ™ extraction process as described (5), and one was extracted manually using the organic extraction and ethanol precipitation process as described in the VDFS procedure manual. The cuttings that were extracted using both the manual and robotic DNA IQ™ System methods were pre-heated at 56°C in 100 μL of DNA IQ™ Lysis buffer for 30 min. After incubation of the samples from all three extraction methods, holes were punched in the depressed lids and the samples were spun for 5 min at 12,000 rpm with cuttings in their respective lids to remove all the liquid from the cutting.

The sensitivity study was repeated to test the effect of a smaller elution volume on the sensitivity of the procedure; however, only the BioMek® 2000/DNA IQ™ System was used for DNA extraction. The software method utilized was changed from a 100 μL elution volume to a 40 μL elution volume. Other deviations from the sensitivity study described above were that instead of 5 mm^2 samples removed for each dilution, a 6 mm circular punch was removed to ensure greater consistency in sampling size and the samples were incubated in the DNA IQ™ Lysis buffer at 56°C for 10 min instead of 30 min.

Substrate/Inhibitory Substances Study

A small cutting (approximately 5 mm^2) was made of each of the blood stains on the various substrates (described above). The cuttings were pre-heated at 95°C in 100 μL of the DNA IQ™ Lysis buffer for 30 min. After incubation, holes were punched in the depressed lids and the samples were spun for 5 min at 12,000 rpm with cuttings in their respective lids to remove all the liquid from the cuttings. Afterwards, the lysates were loaded into the 96 deep well plate and loaded onto the BioMek® 2000 robot for extraction using the DNA IQ™ System.

Environmental Study

Liquid blood smaples from three employees at the VDFS were placed onto Whatman blood stain cards and subjected to different environmental conditions (described above). Samples were collected after incubation and a 5 mm^2 portion removed from each sample for DNA extraction. The sample cuttings were placed into 150 μL of the DNA IQ™ Lysis buffer and incubated for 30 min at 95°C. Afterwards, the cuttings were placed into the depressed lids, after holes had been punched into the lids, and the samples centrifuged for 5 min at approximately 12,000 rpm to remove all the liquid from the cuttings. Lysates were then transferred to a 96 deep well plate and placed onto the robot deck for BioMek® 2000/DNA IQ™ System extraction.

Tissue Extraction Study

A variety of tissue types including pelvis bone, rib bone, brain, heart, liver, blood and muscle were extracted using the BioMek® 2000/DNA IQ™ System. Not all tissue types were available from each source. A small portion of each tissue type was removed using a sterile scalpel (an approximately 4 mm diameter semicircle), placed into 1.5 mL tubes and pre-incubated at 56°C for two hours in a 1X $CaCl_2$ buffer (4X stock: 200 mM Tris PH 8.0, 40 mM $CaCl_2$) containing Proteinase K at a final concentration of 1.8 mg/mL. After incubation, the tubes were spun at approximately 12,000 rpm for 5 min prior to loading the cell lysates directly into a deep well plate, taking care not to disturb the pellet of undigested material at the bottom of the tube.

Results and Discussion

Contamination Studies

The initial testing of the BioMek® 2000/DNA IQ™ System focused on whether the open well, robotic system was suitable for forensic casework samples. For such a robotic system to be applicable to the extraction of evidentiary samples, contamination must be eliminated as a potential problem. Not only can the DNA from casework samples derive from multiple sources, but there may only be a miniscule quantity of biological material available, so the DNA yield is crucial when very little material is available. The contamination tests were repeated multiple times and were varied in an effort to detect minor signals and identify potential sources of contamination. The first two contamination tests, using the "Zebra Stripe" plate format, alternated columns of samples containing relatively concentrated sources of DNA (lysates from blood stains), with columns containing reagent blanks, in a striped pattern across the entire plate for a total of 80 samples and the original version of the software method was employed. This original method involved an initial shaking step with samples placed in a shallow 96 well Greiner plate as well as manual lysis buffer and resin addition steps. Minor contamination was detected in at least one of the reagent blanks and one of the blood stain lysate samples (a faint extra band was visible). The test was repeated and contamination was detected in four of the blood stain lysate samples (additional bands observed), but all the reagent blanks were apparently free of contaminating DNA (data not shown). After these observations of contamination, the software method was modified by Promega scientists to remove the initial shaking step and replace it with a pipetting step for mixing. Samples now could be loaded into a 96 deep well plate instead of the shallow Greiner plate. Simultaneously with these changes, the addition of the lysis buffer and resin was incorporated into the robotic extraction process, eliminating that as a manual step and further reducing the amount of "hands on" time spent preparing samples. Utilizing the newly modified method, the Zebra Stripe test was repeated with no detectable contamination in either the reagent blanks or the lysate samples (data not shown). After the contamination test using the modified method was performed with no detectable contamination, all subsequent extractions were performed using the modified method.

A total of 168 samples, including reagent blanks, were extracted using the "Checkerboard" test for contamination. The Checkerboard test alternated wells containing blood stain lysates, with reagent blank wells, across the plate in a checkerboard pattern. This provided a very sensitive assay to detect both row to row and column to column contamination. Initially, 128 samples were extracted using the Checkerboard plate format. All blood stain samples provided accurate, single source PowerPlex® 1.1 STR profiles, with no evidence of extraneous bands and the reagent blank wells were free of any PCR products (Fig. 3). The Checkerboard test was repeated with samples containing very concentrated sources of DNA in an effort to define the limitations of the open well, robotic extraction platform. Four sets of tissue samples (sets G, N, O, and H) from the following tissue types: brain, heart, pelvis bone, rib bone, liver, and muscle, were extracted using the Checkerboard test. The tissue samples ranged in concentration from 8 ng/μL (a total of 800 ng

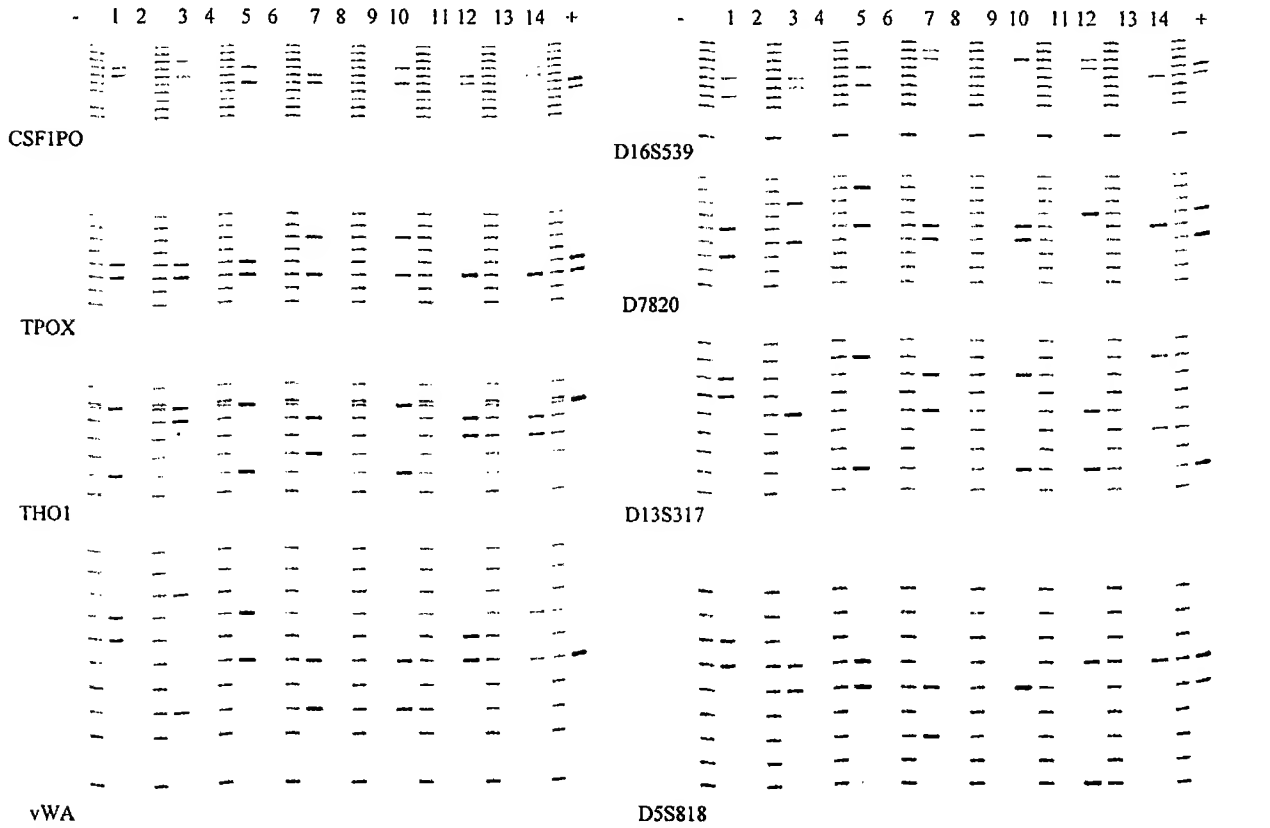

FIG. 3—*PowerPlex® 1.1 System polyacrylamide gel images displaying results from the Checkerboard contamination study. Loci are indicated below and to the left of each allelic ladder. The left panel contains the 585 nm scan and the right panel contains the 505 nm scan. Key: − = negative control, + = positive control (9947A), numbers 1–14 refer to sample numbers.*

of eluted DNA) to less than 0.03 ng/μL (3 ng of eluted DNA) per well. All of the empty reagent blank wells were devoid of any PCR fragments (data not shown). Sample sets G, N, and O displayed clean single source DNA profiles, consistent with one donor for all the tissue types of each sample set. Two of the samples from sample set H provided very weak partial profiles and displayed extra bands that were consistent with the extraneous DNA originating from sample set O. Another sample from set H provided a 6 locus partial profile also displaying extra bands that were consistent with the extraneous DNA originating from sample set O. Both sample set O and H had been acquired several years previously and had been used extensively for student training, while sample sets G and N had been acquired within the past year and had not been used extensively for training purposes. It is unclear whether the mixed DNA samples from sample set H were due to the robotic extraction process or due to repeated use as training samples. However, given that all the reagent blank wells were devoid of any contaminating DNA and that each sample well was surrounded by blank wells, it seems unlikely that the robotic extraction process was the source of the contamination. Thus, the limitations of the open well robot platform for very concentrated sources of DNA were not defined.

Differential Extraction Studies

Initial experiments with mixed stains, consisting of sperm and non-sperm cells, demonstrated that the BioMek® 2000/DNA IQ™ System could complete the DNA extraction once the sperm cells were separated from the non-sperm cells (data not shown). To prepare the mixed stain samples for the robot, the non-sperm cells were lysed open using a digest buffer with Proteinase K, but lacking Dithiotheitol (DTT), as described in Materials and Methods. It was at the point at which a sperm fraction and a non-sperm fraction were generated, that the entire intact sperm cell pellet and the non-sperm (epithelial) cell lysate fractions were loaded into a 96 deep well plate for robotic extraction. A study was performed, which measured the DNA yields from vaginal swabs donated by three different volunteers. Different semen dilutions were placed onto the vaginal swabs and 1/2, 1/4, and 1/8 portions removed from the swabs for differential extractions in order to simulate the standard portions utilized for casework analysis. The epithelial cells were lysed (as described in Materials and Methods) and once the lysis was completed, the sperm cells pelleted and the epithelial cell fraction placed into another tube. A 100 μL aliquot of the epithelial cell lysate from each tube was loaded into a 96 deep well plate for robotic extraction. DNA yields from the epithelial cell lysates were estimated. Data were separated into the 1/2, 1/4, and 1/8 swab sets and averages ($n = 12$) as well as standard deviations calculated (Fig. 4). Even when only 1/8 of a swab was used, an average of approximately 70 ng total of non-sperm DNA was obtained.

A study was performed which compared the performance, measured by DNA yield and quality of STR data, for mixed stain samples whose extraction was completed by the BioMek® 2000/DNA IQ™ System and those completed manually using the organic extraction and ethanol precipitation procedure. Semen dilutions were placed in duplicate on the vaginal swabs taken from five different female volunteers as described in Materials and Methods. The semen dilution series, 1:10, 1:100, 1:1,000 and 1:10,000, was placed onto the swabs in duplicate for the first three vaginal swab donors, creating three dilution series for three mock "couples." The fourth vaginal swab donor had the dilution series, 1:10, 1:100, 1:200 and 1:400, placed onto the swabs and the fifth vaginal swab donor had the dilution series, 1:100, 1:200, 1:400 and 1:800, placed onto the swabs.

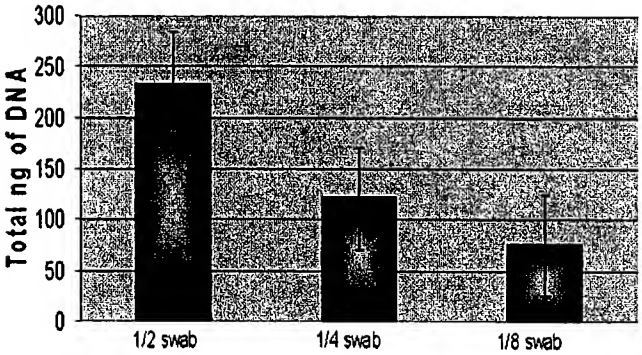

FIG. 4—*Graph depicting epithelial cell DNA yields from differential extractions completed using the BioMek® 2000/DNA IQ™ System. Only 100 μL out of 500 μL of the epithelial cell lysate were loaded into the 96 deep well plate for robotic extraction. N = 12 for each of the three data sets. Error bars indicate the degree of standard deviation from the mean.*

Swabs from each mock "couple" set were cut into 1/2, 1/4, and 1/8 portions, in duplicate, to simulate typical portions used for casework. Then the cuttings were subjected to an epithelial cell lysis, after which the epithelial cell fractions were removed and set aside. The sperm cells were pelleted, rinsed with wash buffer three times and the entire wash buffer removed except for approximately 50 μL at the bottom of each tube. At that point, the tubes were split into two equivalent sets, with one set of sperm pellets and epithelial lysates delivered to students for the first two mock "couple" sets and casework examiners for the last three. The corresponding set of samples for each of the "couple" sets was loaded into the 96 deep well plate for extraction using the BioMek® 2000/DNA IQ™ System. After DNA extraction, all DNA samples were quantitated then typed for the PowerPlex® 1.1 System loci.

The first two comparisons between mock "couple" samples extracted manually and by the BioMek® 2000/DNA IQ™ System showed a marked superiority in performance by the robotic platform. In fact, the robotically extracted samples demonstrated full sperm fraction PowerPlex® 1.1 profiles at the 1:100 semen dilution for the 1/2, 1/4, and 1/8 portions, whereas the manually extracted samples displayed locus drop-out even for some of the 1:10 semen dilutions for both mock "couple" sets (data not shown). These initial two mock "couple" sample sets were manually completed by less experienced students in the laboratory, thus the large discrepancy in performance may have in part been due to the inexperience of the scientists. To address this factor, all following mock couple sample sets were completed by experienced casework examiners.

The third mock couple sample set, also comprised of semen dilutions 1:10, 1:100, 1:1,000 and 1:10,000, displayed full PowerPlex® 1.1 System profiles for both the robotic and manually extracted samples up to semen concentrations at 1:100. Both manually and robotically extracted samples failed to display much beyond an occasional semen donor allele at one or two loci at the 1:1,000 dilution (data not shown). However, the robotically extracted samples (all swab portions) demonstrated detectable sperm fraction DNA yields at the 1:100 semen dilution, whereas the manual organically extracted samples displayed no detectable DNA as measured using the same QuantiBlot (data not shown). To try to better define the semen dilution at which the robotic and manually extracted samples can no longer produce a full PowerPlex® 1.1 profile, the fourth mock "couple" set employed the following dilution series: 1:10, 1:100, 1:200 and 1:400. Even though a different examiner completed the organic extraction, the results were virtually identical to the third mock couple set in that both robotic and manual

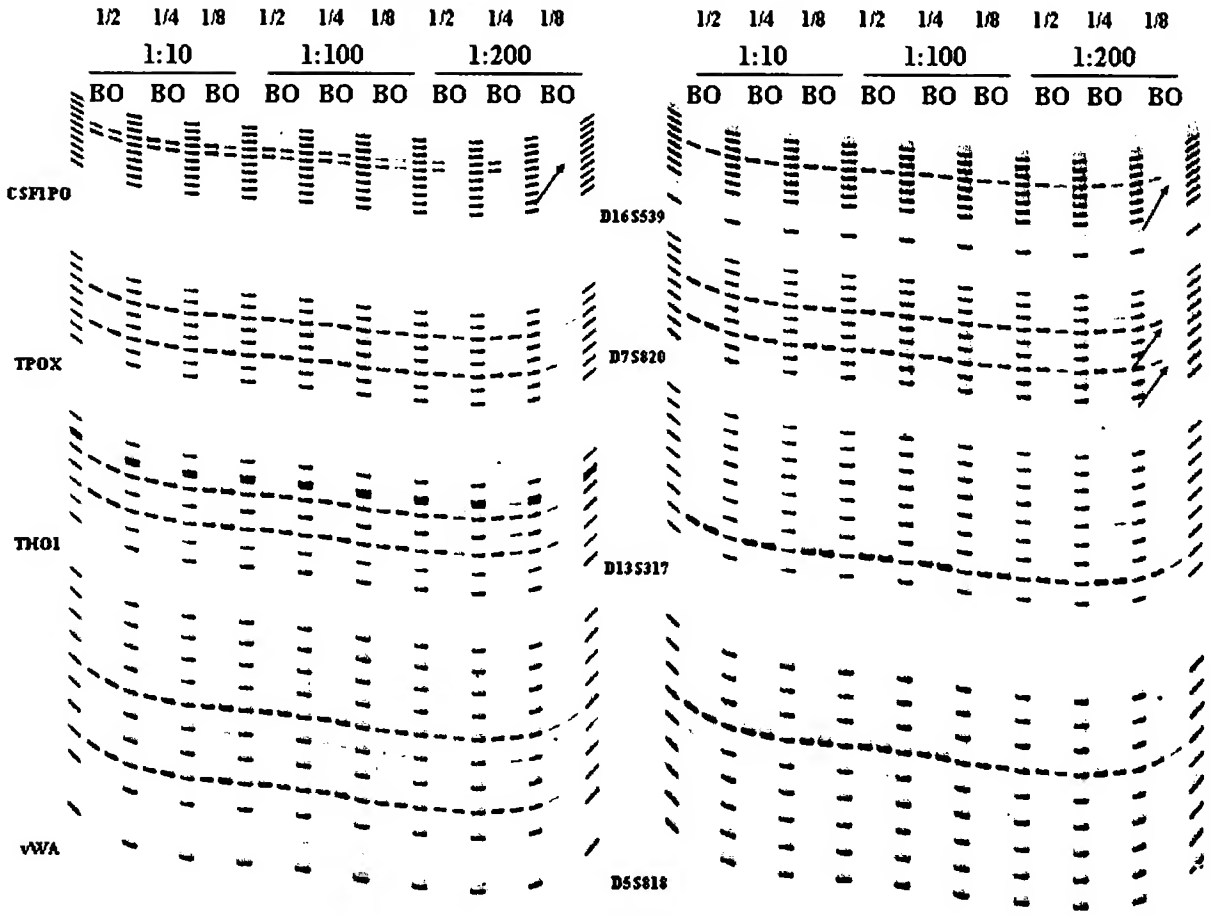

FIG. 5—*PowerPlex® 1.1 System gel images depicting the sperm fraction data obtained from differential extraction samples. The BioMek® 2000/DNA IQ™ System extracted samples are adjacent to the comparable differential extraction samples completed using the manual, organic extraction procedure. The left panel contains the 585 nm scan and the right panel contains the 505 nm scan. Loci are indicated to the left and below each allelic ladder. 1/2, 1/4, and 1/8 refer to the swab portion extracted. 1:10, 1:100 and 1:200 refer to the semen dilutions placed onto the swabs. B = BioMek® 2000/DNA IQ™ System extracted samples. O = manual, organically extracted samples. Arrows point to locus dropout and signal reduction at particular loci.*

extraction methods provided full PowerPlex® 1.1 System profiles to the 1:100 semen dilution for all swab portions, but the robotically extracted samples measured a greater DNA yield than the manually extracted samples at that semen dilution (data not shown). At the 1:200 dilution, the greater yields generated by the BioMek® 2000/DNA IQ™ System were evident (Fig. 5). Arrows point to locus drop-out for the CSF1PO locus and decreased signal at the D16S539 and D7S820 loci for the manual organically extracted sample at the 1/8 swab portion. The robotically extracted sperm fraction sample at the 1:200 semen dilution and the 1/8 swab portion still provided a full PowerPlex® 1.1 System profile.

At the 1:400 dilution, full PowerPlex® 1.1 System profiles were obtained for the robotically extracted sample, for all swab portions. For the manually extracted sample, allelic and locus drop-out was evident at both of the 1/4 and 1/8 swab portions, as well as a decreased signal for some loci. However, the 1/2 swab portion provided a full profile. Both the robotically and manually extracted samples displayed carry-over DNA from the epithelial cell fractions, particularly with the 1/4 and 1/8 swab portions (data not shown).

The final mock sexual assault couple utilized semen dilutions at 1:100, 1:200, 1:400 and 1:800. The results for these samples were less consistent than the results described above. While the BioMek® 2000/DNA IQ™ System extracted samples displayed full sperm DNA profiles at semen dilutions to 1:200, at the 1:400 dilution allelic drop-out was observed for the 1/2 swab and 1/4 swab portions and both allelic and locus drop-out was observed for the 1/8 swab portion (data not shown). For the manually extracted samples, the results varied. Locus drop-out was observed in the DNA samples for all swab portions at the 1:100 semen dilution and at the 1:200 dilution for the 1/2 and 1/8 swab portions. Full PowerPlex® 1.1 profiles were obtained for the 1/4 swab at the 1:200 dilution, the 1/2 swab at the 1:400 dilution and for the 1/4 swab samples at both the 1:200 and 1:400 semen dilutions. At the 1:400 semen dilution, 1/8 swab, no typing results were obtained. Thus, at the 1:400 semen dilution, 1/4 swab sample, more STR data were obtained for the manually extracted sample than for the nearly identical sample that was robotically extracted. At the 1:800 dilution, the BioMek® 2000/DNA IQ™ System extracted samples generated partial profiles for all swab portions, with all samples displaying allelic drop out. The manual organically extracted samples (all swab portions) displayed both allelic and locus drop out (data not shown). Samples extracted using both methods displayed prominent epithelial cell fraction STR products.

More STR data were obtained for the dilute semen samples that were robotically extracted than for the manually extracted samples. One possible explanation for the inconsistency of these data with the previous datasets is that much more carry-over epithelial cell DNA was observed in the sperm fractions than with the previous mock

couple experiments (personal observations). This could potentially impact on the efficiency of sperm DNA typing when little sperm DNA is available to compete with the epithelial cell DNA for STR primer hybridization.

Overall, the samples extracted using the BioMek® 2000/DNA IQ™ System provided more sperm DNA STR data when semen dilutions were 1:100 or greater than the manual organically extracted samples. In accordance with the STR data, the sperm DNA yields, when detectable, were generally greater for the BioMek® 2000/DNA IQ™ System extracted samples than the manually extracted samples when semen dilutions were 1:100 or greater.

Sensitivity Study

A comparative sensitivity study was performed that evaluated the DNA yields and PowerPlex® 1.1 data generated from DNA obtained using the BioMek® 2000/DNA IQ™ System, the manual DNA IQ™ System and the manual organic/ethanol precipitation methods. Blood stain dilution series (neat, 1:10, 1:100, 1:1,000 and 1:10,000) were made using liquid blood samples from three volunteers, approximately 5 mm^2 was cut from each sample of the dilution and the DNA extracted using the three different methods. Samples extracted using the BioMek® 2000/DNA IQ™ System and the manual DNA IQ™ System were eluted in a final volume of 100 μL of DNA IQ™ System elution buffer. Organically extracted samples were reconstituted in a final volume of 100 μL of TE^{-4} in order to directly compare DNA yields.

While yields at the neat, 1:10 and 1:100 dilutions were similar for all three methods (either the same, or generally within a factor of 2, data not shown), differences in yield were clearly evident at the 1:1,000 dilution. At this dilution, none of the extraction methods could reliably produce DNA yields large enough to be detected by the QuantiBlot. However, more complete PowerPlex® 1.1 System profiles (at least 6 out of 8 possible loci) were produced for samples at the 1:100 dilution extracted with the BioMek® 2000/DNA IQ™ System and the manual DNA IQ™ System than for the manual organically extracted samples (data not shown). Moreover, at the 1:1,000 dilution, both the BioMek® 2000/DNA IQ™ System and the manual DNA IQ™ System extracted samples displayed at least some STR data (from 1 to 8 loci out of 8 possible loci), while the manual organically extracted samples displayed no STR typing data for samples from all three volunteers. At the 1:10,000 dilution, only one sample, extracted using the BioMek® 2000/DNA IQ™ System, displayed STR results at two loci. The remaining samples for all three extraction methods produced no results at the 1:10,000 dilution.

The sensitivity of the BioMek® 2000/DNA IQ™ System was further improved upon by reducing the elution volume from 100 μL to 40 μL. The same blood stain dilution series samples were used as described above, however, this time instead of taking 5 mm^2 samples, 6 mm diameter circular punches were taken. All blood stain punches were extracted using the BioMek® 2000/DNA IQ™ System and DNA was either eluted into a 100 μL volume or a 40 μL volume, then typed using the PowerPlex® 16 BIO System. Not only were complete PowerPlex® 16 BIO System profiles produced at the 1:100 dilution for all three volunteers at the 40 μL elution volume, but many loci were typed at the 1:1,000 dilution (as many as 12 loci for one of the samples) and even a few alleles detected for one of the samples at the 1:10,000 dilution (Table 1). What is remarkable is, that when tested for the presence of blood, the 1:1,000

TABLE 1—*Sensitivity study comparing samples eluted in 100 μL versus 40 μL.*

Sample # (elution vol.)	DNA dilution	FGA	TPOX	D8	vWA	Amel	Penta E	D18	D21	TH01	D3	Penta D	CSF	D16	D7	D13	D5
1 (100 μL)	neat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1	1:10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1	1:100	+	−	+	+	+	+	+	+	−	+	+	+	+/−	+	+	+
1	1:1,000	−	−	−	+/−	−	−	−	−	−	−	−	−	−	−	+/−	+
1	1:10,000	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
1 (40 μL)	neat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1	1:10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1	1:100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1	1:1,000	+	−	+	−	−	−	+/−	+	−	−	−	+/−	+/−	−	+	+
1	1:10,000	−	−	−	−	−	+/−	+/−	−	−	−	−	−	−	−	+	−
2 (100 μL)	neat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	1:10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	1:100	+	−	+	+	+	+	+	+	+	+	+	+/−	+	+	+	+
2	1:1,000	−	+/−	−	+/−	−	−	−	+	−	−	−	−	−	−	+	−
2	1:10,000	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2 (40 μL)	neat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	1:10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	1:100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	1:1,000	+	+	+	+/−	+	+	+	+	−	+/−	−	+/−	+	+	+	+
2	1:10,000	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3 (100 μL)	neat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	1:10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	1:100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	1:1,000	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	1:10,000	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3 (40 μL)	neat	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	1:10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	1:100	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	1:1,000	+/−	+/−	−	−	−	+/−	+/−	+	−	+	−	−	+/−	+	+	+
3	1:10,000	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

NOTE: + = complete DNA type, +/− = allelic drop-out, − = no DNA type.

TABLE 2—*PowerPlex® 16 BIO System results for substrate inhibitory substances.*

Donor	Description	FGA	TPOX	D8S1179	vWA	Amel.	Penta E	D18S51	D21S11
1	Hand soap	+	+	+	+	+	+	+	+
1	Carpet	+	+	+	+	+	+	+	+
1	Blk. underwear	+	+	+	+	+	+	+	+
1	Blue Jeans	+	+	+	+	+	+	+	+
1	Contr. foam	+	+	+	+	+	+	+	+
1	Dirt	+	+	+	+	+	+	+	+
1	Canvas	ND	ND	ND	ND	ND	ND	ND	ND
1	Sanitary bag	+	+	+	+	+	+	+	+
1	Hose	+	+	+	+	+	+	+	+
1	Motor oil	+	ND	+	+	+	+	+	+
1	Hand cream	+	ND	+	+	+	+	+	+
2	Motor oil	+	+	+	+	+	+	+	+
2	Hand cream	+	+	+	+	+	+	+	+
2	Hand soap	+	+	+	+	+	+	+	+
2	Canvas	+	+	+	+	+	+	+	+
2	Blue jean	+	+	+	+	+	+	+	+
2	Contr. foam	+	ND	+	+	+	ND	+	+
2	Carpet	+	+	+	+	+	+	+	+
2	Blk. underwear	+	+	+	+	+	+	+	+
2	Sanitary bag	+	ND	ND	+	ND	ND	ND	ND
2	Hose	+	+	+	+	+	+	+	+
2	Dirt	+	+	+	+	+	+	+	+

Donor	Description	TH01	D3S1358	Penta D	CSF1PO	D16S539	D7S820	D13S317	D5S818
1	Hand soap	+	+	+	+	+	+	+	+
1	Carpet	+	+	+	+	+	+	+	+
1	Blk. underwear	+	+	+	+	+	+	+	+
1	Blue Jeans	+	+	+	+	+	+	+	+
1	Contr. foam	+	+	+	+	+	+	+	+
1	Dirt	+	+	+	+	+	+	+	+
1	Canvas	ND	ND	ND	ND	ND	ND	ND	ND
1	Sanitary bag	+	+	+	+	+	+	+	+
1	Hose	+	+	+	+	+	+	+	+
1	Motor oil	+	+	+	+	+	+	+	+
1	Hand cream	+	+	+	+	ND	+	+	+
2	Motor oil	+	+	+	+	+	+	+	+
2	Hand cream	+	+	+	+	+	+	+	+
2	Hand soap	+	+	+	+	+	+	+	+
2	Canvas	+	+	+/−	+	+	+	+	+
2	Blue jean	+	+	+/−	+	+	+	+	+
2	Contr. foam	+	+	ND	ND	ND	+	+	+
2	Carpet	+	+	+	+	+	+	+	+
2	Blk. underwear	+	+	+	+	+	+	+	+
2	Sanitary bag	ND	ND	ND	ND	ND	+	+	+
2	Hose	+	+	ND	+	+	+	+	+
2	Dirt	+	+	+	+	+	+	+	+

NOTE: 1 = sample donor one, 2 = sample donor two, + = full DNA type, +/− = partial DNA type, ND = No Data, Blk. Underwear = black underwear, Contr. Foam = contraceptive foam.

dilution sample produced an inconclusive result using the combined Phenolphthalein-Tetramethylbenzidine test and the 1:10,000 dilution sample produced a negative result (personal observations). The data suggest that overall DNA yields were not reduced when the 40 μL elution volume was used, but instead the DNA samples were more concentrated.

Substrate/Inhibitory Substances Study

A substrate/inhibitory substances study was performed not only to determine if particular substrates introduced inhibitors which are not removed during the BioMek® 2000/DNA IQ™ robotic extraction process, but also to elucidate whether particular substances might interfere with the binding of the DNA to the silica coated paramagnetic resin. Blood was deposited on the different substrates, as well as substrates with possible inhibitory substances on them. The samples were extracted and typed for the PowerPlex® 16 BIO System loci. The STR data obtained are summarized in Table 2. No incorrect DNA profiles were obtained; however an STR profile was not obtained for one sample from a blood donor, which had been deposited on synthetic canvas. This may be due to the method of extraction [Materials and Methods section, Allan Tereba (Promega Corp.), personal communication] since, at the time the samples were extracted, the cuttings were pre-heated at 95°C in the DNA IQ™ Lysis buffer which can melt synthetic material. Current procedure calls for a 56°C incubation for samples, except for blood deposited on FTA paper. This is probably also a valid explanation for the generation of a partial profile from the sanitary bag samples for one of the blood donors and locus drop-out for a hosiery sample from one of the blood donors. Motor oil, hand cream, and the contraceptive foam on cotton also produced partial profiles for one or the other, but not both, of the blood donors. This might be due to an interference of the contaminating substances with the extraction method

employed as the DNA yields were slightly lower than with control samples (data not shown). Further tests must be performed in order to elucidate whether oily substances interfere with the BioMek® 2000/DNA IQ™ System DNA extraction process.

Environmental Study

Blood samples subjected to different environmental conditions (room temperature, 37°C, 56°C, 80°C, exposed to UV light, and moist at room temperature) provided full PowerPlex® 16 BIO profiles at one day and one week, however, at one month, the 56°C and the 80°C samples displayed allelic drop-out and locus drop-out at some loci (data not shown). At three months, a more substantial loss of DNA typing information, both allelic and locus drop-out at many of the loci, was observed at both the 56°C and 80°C temperatures. The loss of DNA typing information from the 56°C and 80°C samples followed a predictable pattern in that information was lost at the largest loci first, which would be consistent with the DNA that was becoming progressively more degraded. What was less expected was the observation that samples kept moist at room temperature for three months displayed a loss of DNA typing data at vWA, TH01 and D3S1358, which are some of the smallest molecular weight loci. Even at the one month and three month incubation time points, samples that were incubated at room temperature, 37°C, and exposed to sunlight, provided full and accurate PowerPlex® 16 BIO profiles (data not shown).

Tissue Extraction Study

A study was performed to evaluate the efficacy of tissue sample extraction using the robotic system. A small portion of each tissue type was removed and placed into a Proteinase K containing buffer as described in Materials and Methods prior to loading onto the robot for BioMek® 2000/DNA IQ™ System DNA extraction. Once extracted, samples were typed for the PowerPlex® 1.1 System loci. Initially, sets of different tissue samples from two different donors (O and H) were extracted and typed. Set O contained muscle, rib bone, heart, liver and blood. Set H contained muscle, heart, brain and liver. The tissues successfully typed at all loci for sample set O were heart, muscle, and blood, with a six locus, partial profile obtained for liver (data not shown). Sample set H provided a full profile only from the muscle sample and partial profiles from brain, liver and blood, with no profile from the heart tissue. None of the extracted samples from set H displayed detectable DNA using the QuantiBlot method. Both sample sets had been extensively used for training over a number of years and had been frozen and thawed multiple times. This may have led to extensive degradation. The DNA profiles obtained were consistent within a sample set.

Two other tissue sample sets (sets G and N) were extracted using the BioMek® 2000/DNA IQ™ System as part of the final contamination test. Set G consisted of pelvis bone, brain, heart, liver and muscle and set N consisted of pelvis bone, rib bone, brain, liver, muscle and heart. Full PowerPlex® 1.1 System profiles were produced for all tissue samples from each set, except for liver sample of set G which provided a six locus partial profile (data not shown). The DNA profiles for the samples were consistent within a set for both donors and DNA yields from some of the samples were high (800 ng for the brain tissue in set G), demonstrating that tissue can be efficiently extracted using this robotic system (data not shown). However, caution should be employed since samples that are very rich sources of DNA may present more of a contamination hazard for the robotic extraction process. Thus, it is recommended that if tissue, particularly fresh or undegraded, is to be extracted for DNA using the BioMek® 2000/DNA IQ™ System, samples removed for extraction should be no greater than the approximate size of 1/2 a pea or a 5 mm cube.

Conclusion

The use of a robotic platform for the extraction of forensic casework samples presents a real opportunity for the crime laboratory to expedite casework processing as well as reduce backlogs. To realize these potential benefits, the system must be flexible enough to handle all the different types of evidentiary samples encountered by a crime laboratory, pose little to no risk of introducing contamination, be easy to use and truly automated such that the forensic scientist need not attend the robotic system, but instead may pursue other job functions. The BioMek® 2000/DNA IQ™ System meets these criteria.

Nearly 200 samples and reagent blanks were analyzed in the contamination study with no evidence of contamination. The extraction of sexual assault type, mixed stain samples, was partially automated, with the sperm cell lysis step occurring on the deck of the robot in a matter of minutes, saving hours of examiner time. Moreover, the comparative study with the mixed stain samples demonstrated that the BioMek® 2000/DNA IQ™ System not only outperformed the manual DNA extraction in both DNA yields of sperm DNA when the semen was very dilute, but also the consistency of STR results produced. The sensitivity of this extraction process was superior to that of the manual, organic, ethanol precipitation process when three blood dilution series were tested. Additionally, when the elution volume for the robotic system was reduced from 100 μL to 40 μL, DNA types were detectable at many loci at a 1:1,000 blood dilution and even a small number of loci could be detected at a 1:10,000 blood dilution, which is below the detection level of the presumptive test for blood employed by the VDFS DNA laboratory. A variety of substrates and potential inhibitory substances were tested using the BioMek® 2000/DNA IQ™ System and none resulted in an incorrect DNA profile. It is possible that oily substances, such as motor oil, may interfere with the DNA binding to the DNA IQ™ paramagnetic resin and reduce yields. However, further experiments must be performed to elucidate this phenomenon. The environmental study demonstrated that accurate PowerPlex® 16 BIO System loci STR types were produced from samples exposed to a variety of environmental conditions. Although samples maintained at elevated temperatures (56°C and 80°C) for extended periods of time (one month and three months) displayed a loss of many of the larger STR loci, the partial profiles obtained were consistent with control sample profiles. Also, samples maintained for three months in a moist environment displayed a loss of some of the smaller loci, yet the partial profiles were still consistent with control STR profiles. Finally, an array of tissue samples was successfully extracted by the robotic system and the STR profiles consistent within the sample sets. Tissue may present more of a problem than other evidentiary samples since it is characteristically a very dense source of DNA. Caution must be employed not to overwhelm the binding capacity of the paramagnetic resin and create potential for contamination. The BioMek® 2000/DNA IQ™ System provides an adaptable, accurate and useful platform for the extraction of evidentiary casework samples.

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